

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 2

Please amend the subject application as follows:

In the Specification:

Please replace the paragraph on page 10, lines 2-5 with the following amended paragraph:

--Figures 15A-15D:

DNA sequence (SEQ ID NO: 128) containing promoter elements from nucleotide -1 to nucleotide -3017. -1 is upstream of start site of PSM.--

Please replace the paragraph on page 10, lines 7-8 with the following amended paragraph:

--Figures 16:

Potential binding sites on the PSM promoter fragment[[.]]: AP1 (SEQ ID NO:103), E2-RS (SEQ ID NO:104), GHF (SEQ ID NO:105), JVC repeat (SEQ ID NO:106), NfκB (SEQ ID NO: 107), uteroglobi (SEQ ID NO: 108), IFN (SEQ ID NO: 109).--

Please replace the paragraph on page 10, lines 13-23 with the following amended paragraph:

--Figure 18:

Comparison between PSM and PSM' cDNA. Sequence of the 5' end of PSM cDNA (32) is shown. Underlined region (beginning at nucleotide 115 and continuing to nucleotide 380) denotes nucleotides which are absent in PSM' cDNA but present in PSM cDNA. Boxed region

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 3

represents the putative transmembrane domain of PSM antigen. *Asterisk denotes the putative translation initiation site for PSM' (SEQ ID NO: 91, SEQ ID NO: 102).--

Please replace the paragraph on page 13, lines 29-36 with the following amended paragraph:

--Figure 32A-32C:

Nucleic Acid of PSM genomic DNA is read 5 prime away from the transcription start site: the number on the sequences indicates the nucleotide upstream from the start site. Therefore, nucleotide #121 is actually -121 using the conventional numbering system (SEQ ID NO: 39).--

Please replace the paragraph on page 15, lines 1-2 with the following amended paragraph:

--Figure 46A-46D:

Intron 1F: Forward sequence (SEQ ID NO: 92).--

Please replace the paragraph on page 15, lines 4-5 with the following amended paragraph:

--Figure 47A-47E:

Intron 1R: Reverse Sequence (SEQ ID NO: 93).--

Please replace the paragraph on page 15, lines 7-8 with the following amended paragraph:

--Figure 48A-48C:

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 4

Intron 2F: Forward Sequence (SEQ ID NO: 94).--

Please replace the paragraph on page 15, lines 10-11 with the following amended paragraph:

--Figure 49A-49C:

Intron 2R: Reverse Sequence (SEQ ID NO: 95).--

Please replace the paragraph on page 15, lines 13-14 with the following amended paragraph:

--Figure 50A-50B:

Intron 3F: Forward Sequence (SEQ ID NO: 96).--

Please replace the paragraph on page 15, lines 17-18 with the following amended paragraph:

--Figure 51A-51B:

Intron 3R: Reverse Sequence (SEQ ID NO: 97).--

Please replace the paragraph on page 15, lines 20-21 with the following amended paragraph:

--Figure 52A-52C:

Intron 4F: Forward Sequence (SEQ ID NO: 98).--

Please replace the paragraph on page 15, lines 23-24 with the following amended paragraph:

--Figure 53A-53E:

Intron 4RF: Reverse Sequence (SEQ ID NO: 99).--

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 5

Please replace the paragraph that begins on page 15, line 26 and ends on page 26, line 10 with the following amended paragraph:

--Figure 54: PSM genomic organization of the exon and 19 intron junction sequences (SEQ ID NO: 39). The exon/intron junctions are as follows:

1. Exon/intron 1 at bp 389-390;
2. Exon/intron 2 at bp 490-491;
3. Exon/intron 3 at bp 681-682;
4. Exon/intron 4 at bp 784-785;
5. Exon/intron 5 at bp 911-912;
6. Exon/intron 6 at bp 1096-1097;
7. Exon/intron 7 at bp 1190-1191;
8. Exon/intron 8 at bp 1289-1290;
9. Exon/intron 9 at bp 1375-1376;
10. Exon/intron 10 at bp 1496-1497;
11. Exon/intron 11 at bp 1579-1580;
12. Exon/intron 12 at bp 1643-1644;
13. Exon/intron 13 at bp 1710-1711;
14. Exon/intron 14 at bp 1803-1804;
15. Exon/intron 15 at bp 1894-1895;
16. Exon/intron 16 at bp 2158-2159;
17. Exon/intron 17 at bp 2240-2241;
18. Exon/intron 18 at bp 2334-2335;
19. Exon/intron 19 at bp 2644-2645.--

Please replace the paragraph on page 16, lines 12-14 with the following amended paragraph:

--Figure 55A-55J:
Alternatively spliced PSM (PSM') nucleic acid sequence (SEQ ID NO: 100) and amino acid sequence (SEQ ID NO:101).--

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 6

Please replace the paragraph on page 19, lines 12-13 with the following amended paragraph:

--**Figure 64:** Sequence analysis of microsatellite instability in PSM gene. Genomic (SEQ ID NO:110), LNCaP (SEQ ID NO:110), PC-3 (SEQ ID NO:111), DU145 (SEQ ID NO:112), T4(tumor) (SEQ ID NO:113), N4(paired normal) (SEQ ID NO:114).--

Please replace the paragraph on page 19, line 17 with the following amended paragraph:

--**Figure 66:** Location of microsatellite in PSM gene (SEQ ID NO:115).--

Please replace the paragraph on page 31, lines 28-31 with the following amended paragraph:

-- In one embodiment, peptides Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID [[No.]] NO:35), Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID [[No.]] NO:36) and Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID [[No.]] NO:37) of human PSM antigen are selected. --

Please replace the paragraph on page 31, lines 33-36 with the following amended paragraph:

-- This invention further provides polyclonal and monoclonal antibody(ies) against peptides Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID [[No.]] NO:35), Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID [[No.]] NO:36) and Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID [[No.]] NO:37) of human PSM antigen are selected. --

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 7

Please replace the paragraph which begins on page 41, lines 18 and ends on page 42, line 13 with the following amended paragraph:

-- This method provides a method of detecting in a sample the presence of a nucleic acid encoding an alternatively spliced human prostate-specific membrane antigen which comprises: a) obtaining a suitable sample; b) extracting RNA from the sample; c) contacting the RNA with reverse transcriptase under suitable conditions to obtain a cDNA; d) contacting the cDNA under hybridizing conditions with two oligonucleotide primers, i) the first primer being capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen (SEQ ID NO:1) located immediately 3' of nucleotide 114 of such DNA sequence, with the proviso that the 3' end of the primer does not hybridize to any sequence located 5' of the nucleotide 114, and ii) the second primer being capable of specifically hybridizing to a sequence with in a DNA sequence encoding prostate specific membrane antigen (SEQ ID NO:1) located immediately 5' of nucleotide 381 of such DNA sequence, with the proviso that the 5' end of the primer does not hybridize to any sequence located 3' of nucleotide 381; [[d)]] e) amplifying any cDNA to which the primers hybridize to so as to obtain amplification product; [[e)]] f) determining the size of the amplification product; [[f)]] g) comparing the size of the amplification product to the size of the amplification product known to be obtained using the same primers with a non alternatively spliced human prostate specific membrane antigen, wherein a smaller amplification product is indicative of the presence of the alternatively spliced human prostate-specific

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 8

membrane antigen sample.--

Please replace the paragraph on page 42, lines 19-29 with the following amended paragraph:

-- In one embodiment, the primers are at least 14-25 nucleotides in length. In another embodiment, the primers are at least 15 nucleotides in length. In another embodiment, multiple primers are used. Construction of primers which hybridize and hybridizing conditions are known to those skilled in the art. For example, based on Figure 18 (SEQ ID NO:91), one skilled in the art may construct primers which hybridize to the prostate specific membrane antigen before nucleotide 114 and after nucleotide 381. --

Please replace the paragraph that begins on page 69, line 28 and ends on page 70, line 3 with the following amended paragraph:

-- **Cloning of PSM promoter.** A bacteriophage P1 library of human fibroblast genomic DNA (Genomic [Sysytems] Systems, Inc., St. Louis, MI), was screened using a PCR method of Pierce et al. Primers located at the 5' end of PSM cDNA were used: 5'-CTCAAAGGGGCCGGATTTC-3' (SEQ ID NO:116) and 5' CTCTCAATCTACTAATGCCTC-3' (SEQ ID NO:117). A positive clone, p683, was digested with XhoI restriction enzyme. Southern analysis of the restricted fragments using a DNA probe from the extreme 5' to the Ava-I site of the PSM cDNA confirmed that a 3Kb fragment contains the 5' regulatory sequence of the PSM gene. The 3 kb XhoI fragment was subcloned into pKSBluescript vectors and sequenced using the dideoxy method. --

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 9

Please replace the paragraph on page 72, lines 7-21 with the following amended paragraph:

-- **Polymerase Chain Reaction.** Oligonucleotide primers (5'-CTCAAAGGGGCCGGATTTC-3' (SEQ ID NO:116) and 5'-CTCTCAATCTACTAATGCCTC-3' (SEQ ID NO:117)), specific for the 5' and 3' ends of PSM cDNA were designed to span the cDNA sequence. The reaction was performed in a 50 µl volume with a final concentration of the following reagents: 16.6 mM NH₄SO₄, 67 mM Tris-HCl pH 8.8, acetylated BSA 0.2 mg/ml, 2mM MgCl₂, 250 µM dNTPs, 10 mM β-mercaptoethanol, and 1 U of rTth polymerase (Perkin Elmer, Norwalk, CT). A total of 25 cycles were completed with the following profile: cycle 1, 94°C 4 min.; cycle 2 through 25, 94°C 1 min., 60°C 1 min, 72°C 1 min. The final reaction was extended for 10 min at 72°C. Aliquots of the reaction were electrophoresed on 1% agarose gels in 1X Tris-acetated-EDTA buffer.--

Please replace the paragraph on page 74, lines 29-33 with the following amended paragraph:

-- PSM' antigen, on the other hand, is a 693 a.a. protein as deduced from its mRNA sequence with a molecular weight of 78,000. PSM' antigen lacks the first 57 amino acids present in the PSM antigen (Figure 18) (SEQ ID NO: 102). It is likely the at PSM' antigen is cytosolic.--

Please replace the paragraph that begins on page 82, line 14 and ends on page 83, line 10 with the following amended paragraph:

-- **Polymerase Chain Reaction.** The PSA outer primer sequences are nucleotides 494-513 (sense) in exon 4 and nucleotides

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 10

960-979 (antisense) in exon 5 of the PSA cDNA. These primers yield a 486 bp PCR product from PSA cDNA that can be distinguished for a product synthesized from the possible contaminating genomic DNA.

PSA-494 5'-TAC CCA CTG CAT CAG GAA CA-3' (SEQ ID NO:118)

PSA-960 5'-CCT TGA AGC ACA CCA TTA CA-3' (SEQ ID NO:119)

The PSA' upstream primer begins a nucleotide 559 and the downstream primer at nucleotide 894 to yeild a 355 bp PCR product.

PSA-559 5'-ACA CAG GCC ACC TAT TTC AG-3' (SEQ ID NO:120)

PSA-894 5'-GTC CAG CGT CCA GCA CAC AG-3' (SEQ ID NO:121)

All primers were synthesized by the MSKCC Microchemistry Core Facility. 5 µg of total RNA was reverse-transcribed into cDNA using random hexamer primers (Gibco-BRL) and Superscript II reverse transcriptase (Gibco-BRL) according to the manufactures recommendations. 1 µl of the CDNA served as the starting template for the outer primer PCR reaction. 20 µl PCR mix included: 0.5U Tag polymerase (Promega) Promega reaction buffer, 1.5 mM MgCl₂, 200 µM dNTPs and 1.0 µM of each primer. This mix was then transferred to a Perkin Elmer 9600 DNA thermal cycle and incubated for 25 cycles. The PCR profile was as follows: 94°C x 15 sec., 60°C x 15 sec., and 72°C for 45 sec. After 25 cycles, samples were placed on ice, and 1 µl of this reaction mix served as the template for another 25 cycles using the inner primers. The first set of tubes were returned to the thermal cycler for 25 additional cycles. The PSM outer upstream primer sequences are nucleotides 1368-1390 and the downstream primers are nucleotides 1995-2015, yielding a 67 bp PCR product.

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 11

Please replace the paragraph that begins on page 83, line 12 and ends on page 84, line 6 with the following amended paragraph:

-- PSM-1368 5'-CAG ATA TGT CAT TCT GGG AGG TC-3' (SEQ ID NO: 122)

PSM-2015 5'-AAC ACC ATC CCT CCT CGA ACC-3' (SEQ ID NO: 123)

The PSM inner upstream primer span nucleotides 1689-1713 and the downstream primer span nucleotides 1899-1923, yielding a 234 bp PCR product.

PSM-1689 5'-CCT AAC AAA AGA GCT GAA AAG CCC-3' (SEQ ID NO: 124)

PSM-1923 5'-ACT GTG ATA CAG TGG ATA GCC GCT-3' (SEQ ID NO: 125)

2µl of cDNA was used as the starting DNA template in the PCR assay. The 50µl PCR mix included: 1U Taq polymerase (Boehringer Mannheim), 250 µM dNTPs, 10 mM β-mercaptoethanol, 2mM MgCl₂, and 5 µl of a 10x buffer mix containing 166 mM NH₄SO₄, 67 mM Tris-HCl pH 8.8, and 2mg/ml of acetylated BSA. PCR was carried out in a Perkin Elmer 480 DNA thermal cycler with the following parameters: 94°C x 4 minutes for 1 cycle, 94°C x 30 sec., 58°C x 1 minute, and 72°C x 1 minute for 25 cycles followed by 72°C x 10 minutes. Sample were then iced and 2.5µl of this reaction mix was used as the template for another 25 cycles with a new reaction mix containing the inner PSM primers. cDNA quality was verified by performing control reactions using primers derived from the β-2-microglobulin gene sequence¹⁰ a ubiquitous housekeeping gene. These primers span exons 2-4 and generate a 620 bp PCR product. The sequences for these primers are:

β-2 (exon 2) 5'-AGC AGA GAA TGG AAA GTC AAA-3' (SEQ IN NO:126)

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 12

β -2 (exon 4) 5'-TGT TGA TGT TGG ATA AGA GAA-3' (SEQ IN NO:127) --

Please replace the paragraph that begins on page 113, line 23 and ends on page 117, line 28 with the following amended paragraph:

--Knowledge of the intron exon junctions allows for the selection of primer pairs that cross an intron junction and thus allow the determination of DNA contamination of the RNA preparation, if present. If the intron junction were large it would be unlikely to be amplified with primers, while if the intron junction were small it would still produce a fragment that would be much larger than the predicted fragment size which is based on the cDNA sequence. Thus knowledge of the intron/exon junctions provides a control to determine if the RT-PCR product is contaminated with DNA. Another form of DNA that could also be amplified undesirably if present as a contaminant are pseudo genes, which are intronless forms of the mRNA that reside as DNA but are not expressed as RNA. Thus, optimized primers for detection of PSM mRNA in samples would preferably contain sequences hybridizing across the ~~intron/exon~~ intron/exon junction which are as follows:

EXON 1	Intron 1
1F. strand	
CGGCTTCCTCTTCGG (<u>SEQ ID NO:40</u>)	
cggcttcctcttcgg	taggggggcgcctcgcggag...tatatt ttca (<u>SEQ ID NO:41</u>)
1R. strand	
	...ataaaaagtCACCAA (<u>SEQ ID NO:42</u>)

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 13

EXON 2 Intron 2

2F. strand

ACATCAAGAAGTTCT (SEQ ID NO:43)

acatcaagaagttct caagtaagtccatactcgaag... (SEQ ID NO:44)

2R. strand ...caagtggtcATATATTAAATG (SEQ ID NO:45)

EXON 3 Intron 3

3F. strand

GAAGATGGAAATGAG (SEQ ID NO:46)

gaagatggaaatgag gtaaaatataaataaataaataa... (SEQ ID NO:47)

3R. strand ...TAAAGTTGTGTAGT (SEQ ID NO:48)

EXON 4 Intron 4

4F. strand

AAGGAATGCCAGAGG (SEQ ID NO:49)

aaggaatgccagagg taaaaacacagtgaacaaa... (SEQ ID NO:50)

4R. strand ...agagttgCCGCTAGATCACA (SEQ ID NO:51)

EXON 5 Intron 5

5F. strand

CAGAGGAAATAAGGT (SEQ ID NO:52)

cagaggaaataaggt aggtaaaaattatctctttttt... (SEQ ID NO:53)

5R. strand ...gtgttttctATTTTACGGGT (SEQ ID NO:54)

EXON 6 Intron 6

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 14

6F. strand

GTTACCCAGCAAATG (SEQ ID NO:55)

gttaccagcaatg gtgaatgatcaatccttgaat...(SEQ ID NO:56)

6R. strand ...aaaaaaagtTTATACGAATA (SEQ ID NO:57)

EXON 7

Intron 7

7F. strand

ACAGAAGCTCCTAGA (SEQ ID NO:58)

acagaagctcctaga gtaagtttgtaagaaaccargg...(SEQ ID NO:59)

7R. strand ...aaacacaggttatcTTTTTACCCA (SEQ ID NO:60)

EXON 8

Intron 8

8F. strand

AAACTTTTCTACACA (SEQ ID NO:61)

aaacttttctacaca gttaagagactatataaatttta...(SEQ ID NO:62)

8R. strand ...aaacgtaatcaTTTTCAGTTCTAC (SEQ ID NO:63)

EXON 9

Intron 9

9F. strand

AGCAGTGGAACCAG (SEQ ID NO:64)

agcagtggaaccag gttaaaggaatcgtttgctagca...(SEQ ID NO:65)

9R. strand ...aaagaTGTCTATACAGTAA (SEQ ID NO:66)

EXON 10

Intron 10

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 15

10F. strand

CTGAAAAAGGAAGG (SEQ ID NO:67)

ctgaaaaaggaagg taatacaaacaaatagcaagaa...(SEQ ID NO:68)

EXON 11

Intron 11

11F. strand

TGAGTGGGCAGAGG (SEQ ID NO:69)

agaggtaggttgtaatttgctataatata...(SEQ ID NO:70)

EXON 12

Intron 12

12F. strand

ATCTATAGAAGG (SEQ ID NO:71)

gtagtttcct gaaaaataagaaaagaatagat...(SEQ ID NO:72)

EXON 13

Intron 13

13F. strand

CTAACAAAAGAG (SEQ ID NO:73)

agggcttttcagct acacaaattaaaagaaaaaaag...(SEQ ID NO:74)

EXON 14

Intron 14

14F. strand

GTGGCATGCCCAGG (SEQ ID NO:75)

gtggcatgcccagg taaataaatgaatgaagtttcca...(SEQ ID NO:76)

EXON 15

Intron 15

15F. strand

CTAAAAATTGGC (SEQ ID NO:77)

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 16

aatttgtttgtttcc tacagaaaaacaacaaaaca...(SEQ ID NO:78)

EXON 16

Intron 16

16F. strand

CAGTGTATCATTTG (SEQ ID NO:79)

cagtgtatcatttg gtatgttacccttcctttttcaaatt...(SEQ ID NO:80)

16R. strand ...aaagtcTAAGTGAAAA (SEQ ID NO:81)

EXON 17

Intron 17

17F. strand

TTTGACAAAAGCAA (SEQ ID NO:82)

tttgacaaaagcaa gtatgttctacatatatgtgcatat...(SEQ ID NO:83)

17R. strand ...aaagagtcGGGTTATCAT (SEQ ID NO:84)

EXON 18

Intron 18

18F. strand

GGCCTTTTTATAGG (SEQ ID NO:85)

ggcctttttatagg taaganaagaaaatatgactcct...(SEQ ID NO:86)

18R. strand ...aatagttgGTACAGTAGATA (SEQ ID NO:87)

EXON 19

Intron 19

19F. strand

GAATATTATATATA (SEQ ID NO:88)

gaatattatatata gttatgtgagtgtttatatatgtgtgt...(SEQ ID NO:89) --

Applicants : Warren D. W. Heston, et al.
Serial No. : 10/614,625
Filed : July 2, 2003
Page 17

Please add the Sequence Listing attached hereto as **EXHIBIT C**.